Pine

DEATH OF ROOT TISSUES IN STANDING [LIVE] AND FELLED LOBLOLLY PINES[1]

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Abstract—Recycling tree root components is important in sustaining the productivity of southern pine forests. Death of outer cortical tissues and mortality of short roots is ubiquitous in conifers. Affected tissues lose their starch grains and accumulate secondary products, such as tannins. In this study, 10-year-old loblolly pine trees were cut at the soil surface and sequential samples of roots were collected, fixed, embedded, and sectioned for light microscopy at monthly intervals. Observations showed roots of felled trees were similar to those of standing controls for approximately 5 months. Indicators of cell and tissue death were the disappearance of starch grains, increased tannin accumulation, and decreased staining of nuclei. This pattern of changes was remarkably similar to that of dying cortical cells. The long period (5 months) after felling and before the roots die probably has a significant effect on root microflora and the distribution of nutrients from the decomposition of surface woody debris and root systems.

INTRODUCTION

Loss of biomass from the crowns of loblolly pines (*Pinus taeda* L.) is easy to measure (Kozlowski and others 1991; Sampson and others 1998); loss of below-ground biomass can be harder to sample and measure (Kozlowski 1971, Ruark 1993). Death and rate of root decay are important on many reforestation sites where nutrient supply is marginal for seedling establishment and early tree growth. Nutrient cycling is influenced by how quickly root turnover occurs. Death of root cells was reviewed by Coulter as early as 1900 (Eames and MacDaniels 1947). I used ease of peeling the root cortex to assess the condition of primary roots. Smith (1935), Eames and MacDaniels (1947) and Esau (1953) detailed the function of the root cortex and its relationship to secondary growth. Those anatomical descriptions emphasize the complexity of below-ground biomass loss. Moreover, they suggest that microscopical examination is essential for classifying cortical cells as dead. Medical investigators routinely use a number of cellular traits to determine cell death (Ellis and others 1991, Robbins 1987). Emphasis is placed on the condition of the nucleus when standardized stain schedules are applied to sections of tissue. I applied such schedules to pine root tissues.

My objective was to devise quantitative measurements of cell traits that would precisely define root cell death. After accomplishing this, cell death was induced by tree felling and studied in detail. These two approaches provided a quantitative method for studying below-ground biomass in loblolly pine roots.

SITES

Observations to select methods and cellular traits were made on roots from young (5 to 10 years) loblolly pine stands in the Palustris Experimental Forest (Louisiana), the Homochitto National Forest (Mississippi), and in a Forest Service planting near Laurinburg, NC. A total of 5,476 roots were sectioned and stained for light microscopy.

Experiments to induce root-cell death were conducted in the Palustris Experimental Forest. Treatments imposed in a 10-year-old loblolly pine study area included: (1) a control (no treatment), (2) felling in February and May 1994, (3) girdling at breast height, and (4) pruning lower limbs (leaving the top 1/3 of crown). Root anatomy of 10 trees each of the control and those that were felled in February and May (treatment 2) was evaluated each month for 6 months. Treatments 3 and 4 were applied in May and sampled only 5 months following treatment.

PROCEDURES

Roots were sampled 1 m from the stem to a 20-cm depth for 6 to 10 trees at each site (Walkinshaw 1995). A cross-section of each root <1 cm in diameter was excised and placed unwashed into formalin-acetic acid-alcohol (FAA) (Sass 1951). After 2 to 4 weeks, roots were rinsed with 70 percent ethyl alcohol. Specimens were cut to 1 to 3 mm, dehydrated in ethyl alcohol series, embedded in paraffin and cut into 7- to 10-μm sections. Two or three sections that contained 9 to 18 roots from a single tree were mounted on a slide. Nine slides were prepared for each tree. Several staining schedules were used on root sections during the observation phase: acid fuchsin, Congo red, Giemsa, Groett's methenamine, safranin-aniline blue, toluidine blue, hematoxylin-eosin, Papanicolaou's schedule, and an acid-Schiff schedule (Haas 1980). Only the last three were used during the experimental phase. Root traits were scored as proportions or as real values. Papanicolaou's schedule was read for two slides with two or three sets of roots per slide. I used hematoxylin-eosin stain to verify nuclear viability in cells. Starch and tannin deposits were confirmed using an acid-Schiff schedule (Walkinshaw and Tiaras 1998). Cell traits used as dependent variables in the treatment evaluations are listed in table 1.

RESULTS

Initial Observations

Shedding of the root cortex was first indicated in a large number of cells, distributed at random, by the breakdown of starch grains. Nuclei with changed stain affinity were prominent in most parenchyma and cortical ray cells. Cytoplasm became condensed to a small volume in the cortical cell periphery. These cells appeared net-like with primary cell walls held to each other and to the thin residual of living cortical cells. Nuclear staining as a measure of loss of vitality indicated that death of the cortex shed occurs after the loss of starch grains (Greenberg 1997).

The proportion of roots with shedding was high in collections from 5- and 10-year-old trees in the Palustris Experimental

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[1] Paper presented at the Tenth Biennial Southern Silvicultural Research Conference, Shreveport, LA, February 16-18, 1999.

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Table 1—Cell traits used to evaluate root viability in loblolly pine

Trait	Description
Abnormal cambium	Cambial initials reduced in number or out of alignment. Necrotic derivations present.
Cortex shedding	Cortical cells dead and remain attached or are released into the soil.
Dead root	Cells with ruptured membranes. Tannin adheres to cell walls. Chromatin abnormal. Starch grains may or may not be present.
Nuclear stain	Degeneration (pyknosis) of chromatin. Altered staining throughout the tissues.
Number of	Number of starch-containing plastids per cell viewed at a single focus per cell length at 100 to 500 diameters.
Root diameter	Actual measurement of roots less than 3 mm. Estimates for larger roots. Diameter includes attached shed.
Size of	Size of starch grains scored as 1, 2, or 3 for each cell. Range in actual size was 0.5 to 4.0 microns.
Starch use	Starch grains 50 percent or more degraded.
Tannin	Accumulation of tannin-containing cells in the cortex, rays and inner xylem. Number of cells with accumulation <10 to >1000.

Forest (table 2); I collected root samples in the fall on two sites there. The biomass loss in shed material was 65 to 75 percent of the root as determined by light microscopy.

Mycorrhizal short roots died during cortex shedding. Tannin accumulated at their base and sealed the torn end of the dead short root. New lateral roots often emerged from the dead mycorrhizal short roots.

Table 2—Incidence of shedding in roots of plantation-grown loblolly pines on different sites

Number of roots sectioned	Proportion of roots with cortical shedding
111	0.90 ± 0.06
120	.78 ± .09
126	.80 ± .16
132	.70 ± .12
119	.69 ± .14
112	.73 ± .16

Starch grain degradation varied from 0 to 100 percent in cortical cells of loblolly pine roots (table 3). Values were low for the younger 5-year-old trees and widely different in collections from 10-year-old trees.

Table 3—Variation in use of starch grains in the root cortex in loblolly pines[a]

Number of roots	Proportion of roots with starch utilization
101	0.93
122	.76
079	.41
064	.33
264	.17
258	.01

[a] Largest two samples of roots were taken from 5-year-old trees. Other samples were from 10-year-old trees.

Experimental

An analysis of variance (ANOVA) procedure using ``month after felling" as class gave a probability of p = 0.0001 for each dependent variable. Tukey's studentized range test (Snedecor 1956) indicated significance when comparisons were made of 5-month means and 0-, 1-, 3- and 4-month means, respectively. Means for traits and treatments 5 months after felling are given in table 4. A plot of abnormal nuclei and dead roots 6 months after felling is given in figure 1. Means for other traits from 0 to 6 months after felling are given in figures 2 and 3.

DISCUSSION

Initial Observations

The cortical shedding in roots from felled loblolly pines appears to involve about 75 percent of the primary root biomass. Some of the biomass may be lost with the gradual disappearance of starch grains. Abnormal staining of chromatin in the nucleus indicated depletion of energy and death of the cortical cells. Cell mortality in the formation of shed material was unusual for the low incidence of tannin that accumulated. Wounds in the cortex were unusually few and microbial invasion of cells in the shed material was

Table 4—Cellular traits in sectioned roots sampled five months after installing treatments

Trait	Fell 2/94	Fell 5/94	Girdle	Prune	Control
			Percent of roots		
Nuclei	50.0	40.0	9.0	2.0	0.0
Starch grain (no.)	2.2	2.4	5.2	4.0	10.2
Size of starch	1.0	0.6	1.5	1.2	2.3
Starch use	30.0	10.0	30.0	1.2	76.0
Tannin X 5	70.0	60.0	50.0	20.0	26.0
Cambium dam.	40.0	30.0	0.0	0.0	0.0

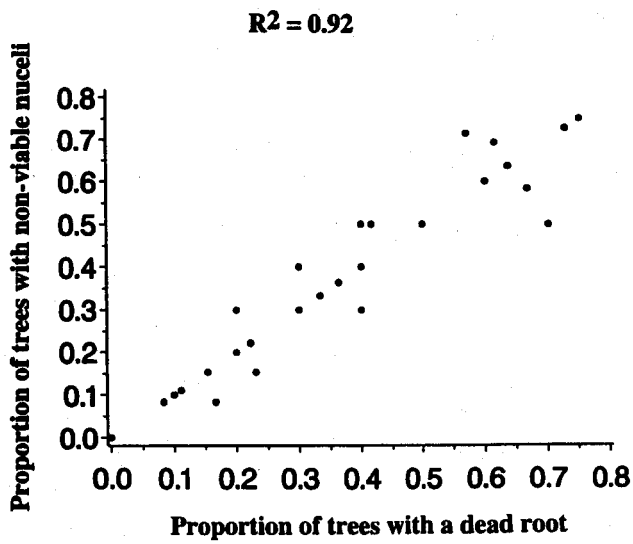

Figure 1—Relationship between nuclear condition and dead roots 5 months after felling.

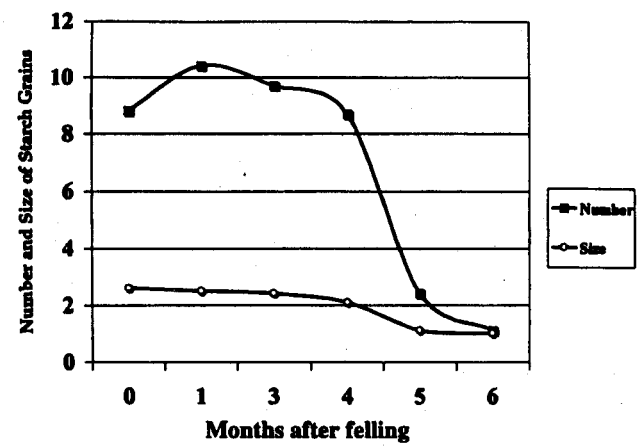

Figure 2—Effect of felling on number and size of starch grains in cortical cells.

Root death was characterized by depletion of starch, abnormal staining of nuclei, and alteration of tannin deposits. Tannin was released from cell vacuoles and increased protein alteration. In roots of felled trees, the appearance of cortical cells after 5 and 6 months was similar to that of cells of cortex shed. Both conditions suggest nutrient starvation. In trees girdled or pruned, root anatomy did not differ from that of the control.

delayed until they were nearly devoid of cytoplasm. These events can be compared to shedding of above-ground plant parts (Kozlowski 1973). Although mycorrhizal root death and shedding occurred simultaneously, considerable tannin accumulated at the base of these short roots. This suggests a more active process than occurred in shed cells. Mycorrhizal roots are not connected to vascular tissue.

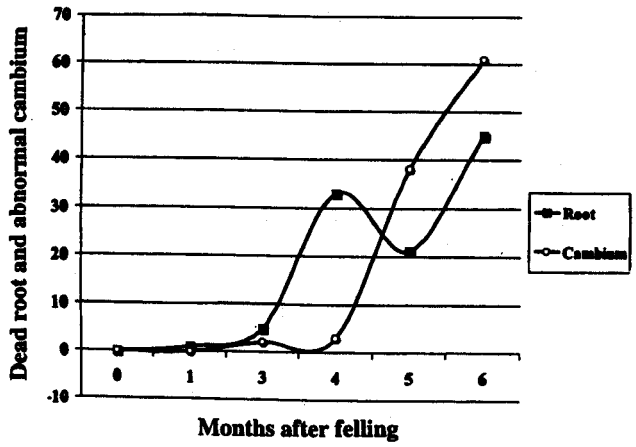

Figure 3—Delayed response of loblolly pine roots to felling.

Lateral roots form only when nuclear division occurs in the vascular zone (Smith and Read 1997); they are, therefore, only temporary structures in the below-ground biomass (Kozlowski 1971).

Experimental

Death of root cells in felled trees occurred in a sequence that has been described for other plant and animal cells (Ellis and others 1991, Greenberg 1997, Robbins 1987). Ample evidence shows that nuclear staining is a reliable indicator of cell death. In pine roots, nuclei are relatively large (8 μm) and easy to classify as active (bright red), quiescent (bluish purple), and dead (gray with black). Abnormal nuclei appeared in large numbers 5 months after felling. This signaled root death.

Microscopical light observations have focused on anatomical details in only a few specimens (Eames and MacDaniels 1947, Esau 1953, Kozlowski and others 1991). This study considered thousands of observations and compared variables and means by standard statistical analysis. When the means of dependent variables were plotted, plot trends were apparent over time. However, the data taken over time are not independent and should be considered with caution. The easiest variable to quantify microscopically was the number of starch grains per cell. However, as cell death approached (5 months after felling), starch grains became so depleted that counts had to be made at 500 diameters. By contrast, nuclei were the same size or had enlarged before death occurred.

Often, silviculturists and pathologists consider below-ground tissue death to occur when trees are felled. However, Bormann (1961) showed that eastern white pine (*Pinus strobus* L.) trees that were not root grafted to intact standing trees were alive one growing season following cutting. Because trees in this study were young, extensive root grafting had not likely occurred (Kozlowski 1971). Three to four months after felling, the roots (small to large) from felled trees were not anatomically different from untreated controls. Pathogenic and saprophytic organisms might increase significantly in roots of freshly felled trees, but unless there is significant root grafting there should be little effect on standing trees. Stand density and tree age may also affect root interaction with microorganisms and the degree of root deterioration, but we did not measure those variables.

CONCLUSIONS

Death of cortical cells and mycorrhizae during the shedding process can cause a loss of 75 percent of the primary root biomass. A lot of starch grains are broken down during shedding, a process that can be reproduced by root starvation resulting from removal of above-ground tissues. Of the variables evaluated, disappearance of starch grains and abnormal nuclei staining are the most reliable when monitoring cell death. Roots of felled trees lived 4 months or longer. The extended life of a felled tree's roots probably will significantly affect microflora. The distribution of nutrients from extended root decay following harvest may affect nutrient availability for forest regeneration.

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